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An Advanced Phase 1/2 Study using an XC-Targeted Gene Therapy Vector for Chemotherapy Resistant Sarcoma

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Abstract

Background: Gene therapy vectors can be delivered directly to the tumor microenvironment (TME) by targeting exposed collagenous (XC) proteins found in abundance at sites of tumor invasion, stroma formation, extracellular matrix remodeling, and neoangiogenesis.

Purpose: To determine the safety and efficacy of an XC-targeted retro vector bearing a cytocidal dominant-negative mutant cyclin G1 gene (Rexin-G) in chemotherapy-resistant sarcoma.

Patients/Methods: Thirty-six patients with metastatic sarcoma received escalating doses of Rexin-G intravenously from 8x10e11 cfu to 48x10e11 cfu/6-week cycle.

Results: No dose-limiting toxicity was observed, and no vector DNA integration, replication-competent retrovirus, nor vector-neutralizing antibodies were detected. Thirty-three patients were evaluable for efficacy analysis. Using RECIST vs. 1.0, at Dose I, 3/6 patients had stable disease; median progression-free survival was 1.2 months, and median overall survival, 3.2 months with a one-year survival rate of 0%. At Dose II-III, 10/14 patients had stable disease; median progression-free survival, 3.8 months, median overall survival, 7.8 months, a one-year survival rate of 28.6% and no 2-year survivors. At Dose IV-V, 8/13 patients had stable disease; median progression-free survival, 4.1 months, median overall survival, 11.5 months, a one-year survival rate of 38.5%, and a 2-year survival rate of 31%. Further, the observed dose-response relationship between Rexin-G dosage and overall survival time was highly significant (p=0.002). A greater proportion of partial responses and stable disease were noted using the PET and CHOI Criteria, suggesting that these tests are more sensitive than merely anatomical assessment in evaluating early responses to Rexin-G. Taken together, these data indicate that Rexin-G is safe, controls tumor growth, and improves overall survival in a dose-dependent manner in chemotherapy-resistant sarcoma.

Keywords: Targeted gene delivery; Sarcoma; Cell cycle; Cancer therapy; Tumor microenvironment

Abbreviations

XC: Exposed Collagenous Proteins; STS: Soft Tissue Sarcoma; U.S: United States of America; USFDA: United States Food and Drug Administration; DNA: Deoxyribonucleic Acid; TME: Tumor Microenvironment; CFU: Colony Forming Units; i.v.: Intravenous; DLT: Dose Limiting Toxicity; MTD: Maximum Tolerated Dose; CTCAE vs. 3.0: Common Terminology Criteria for Adverse Events; AE: Adverse Event/s; OR: Objective Response; RECIST vs.1.0: Response Evaluation Criteria in Solid Tumors Version 1; PET: Positron Emission Tomography; ECOG: Eastern Cooperative Oncology Group; HIV: Human Immunodeficiency Virus; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; Sv40: Simian Virus 40; RCR: Replication Competent Virus; *env:* Envelope; S.P.C: Sant P. Chawla; CT: Computerized Axial Tomography; FDG: 2-deoxy-2-[fluorine-18]fluoro- D-glucose ; PR: Partial Response; SD: Stable Disease; PD: Progressive Disease; CR: Complete Response; SUV: Standard Uptake Value; PFS: Progression Free Survival; OS: Overall Survival; NCSS: Number Cruncher Statistical Systems

Introduction

Soft tissue sarcoma (STS) is a relatively rare neoplasm of mesenchymal origin, which includes a diversity of histological subtypes and occurs with an incidence of about 1% of all adult cancers. The American Cancer Society's projection for the year 2015 is that about 11,930 new cases will be diagnosed in the U.S. and 4,870 Americans will die of soft tissue sarcoma. Surgical resection is still the treatment of choice for localized disease, along with radiation therapy for unresectable sarcomas. However, recurrence rate is high (~50%) and treatment options for metastatic/relapsed STS are limited to anthracycline-based chemotherapy (i.e., doxorubicin), administered alone or in combination with alkylating agents (ifosfamide and/or

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dacarbazine) [1, 2]. Judging from studies conducted over the last 20 years, prognosis for advanced STS has been uniformly poor, with an estimated median survival of only 8 to 13 months [3-5], which falls to 2.0 to 6.6 months from initiation of second-line treatments [2]. Recently, two new drugs for soft tissue sarcoma have been approved by the United States Food and Drug Administration (USFDA). The first drug, pazopanib (*Votrient®*, Novartis Pharma), is a multiple tyrosine kinase inhibitor, which hinders growth factor-mediated signal transduction pathways involved in tumor angiogenesis [6-8]. The second drug, trabectidin/ET-743 (*Yondelis®*, Janssen Biotech, Inc.), is a natural alkaloid, originally isolated from the Caribbean tunicate, *Ecteinascidia turbinata*, which interacts with DNA in a complex manner that interferes with gene transcription and DNA repair [9]. Following the approval of trabectedin in Europe for treatment-resistant STS [10,11] and the results of a pivotal Phase 3 trial [12], which enabled its approval in the United States for liposarcoma and leiomyosarcoma in 2015, there is renewed optimism for improving the quality of life, progression-free survival, and potentially, the overall survival of advanced STS patients that have failed standard therapies [13].

Alternatives to chemotherapy for advanced STS include recent advances in delivering gene therapy vectors directly to the tumor microenvironment (TME), which can be achieved by targeting the pathological exposure of collagenous (XC) proteins caused by tumor invasion, stroma formation, and neoangiogenesis [14,15]. Indeed, the abnormal exposure of XC-proteins within the TME has been utilized to therapeutic advantage with the development of an active tumor-targeting platform that enables efficient delivery of replication-incompetent retroviral vectors to primary and metastatic lesions, while sparing normal tissues [16-18]. Rexin-G, the first, and so far only, targeted injectable gene therapy vector [19] which bears a cytocidal dominant-negative cyclin G1 construct [20], has been previously tested in three Phase 1/2 clinical trials for chemotherapy-resistant sarcoma, pancreatic cancer, and breast cancer, and in one Phase 2 study for chemotherapy-resistant osteosarcoma in the United States [21-23]. In this article, we report on the results of an expanded Phase 1/2 dose-escalation study assessing the safety, toxicity, and potential efficacy of Rexin-G as salvage monotherapy for metastatic sarcoma.

Patients and Methods

Study design

This was an open label, single center, single arm, dose-seeking study that incorporated a modification of the standard Cohort of 3 designs [24] combined with a Phase II efficacy phase. For the Phase 1 part of the study, treatment with Rexin-G comprised 6-week cycles that encompassed 4 weeks of treatment followed by a 2-week rest period. Five dose levels were planned, beginning at 1.0 x 10e11 cfu given by intravenous (i.v.) infusion two times per week. Three patients were to be treated at each dose level, with expansion to 6 patients per cohort if dose limiting toxicity (DLT) was observed in any 1 of the first 3 patients at each dose level. The maximum tolerated dose (MTD) was defined as the highest dose in which 0 of 3 or ≤1 of 6 patients experienced a DLT, with the next higher dose level having at least 2 patients who experienced a DLT. A DLT was defined as any National Cancer Institute Common Toxicity Criteria for Adverse Events version 3 (CTCAE vs. 3) Grade 3, 4, or 5 adverse event (AE) considered possibly, probably, or definitely related to the study drug, excluding the following: Grade 3 absolute neutrophil count lasting <72 hours; Grade 3 alopecia; or any Grade 3 or higher incident of nausea, vomiting, or diarrhea in a patient who did not receive maximal supportive care.

Table 1: Patient Demographics (N = 36).

Age, Years	
Median	48.8
Range	(12.0-70.0)
Gender	
Female	16 (44.0%)
Male	20 (56.0%)
Race	
White	31 (86.1%)
Hispanic	3 (8.3%)
African-American	1 (2.8%)
Asian	1 (2.8%)
Disease Stage	
Metastatic	35 (97.2%)
Non-metastatic	1 (2.8%)
Performance Score	
1	36 (100.0%)
# Previous Chemotherapy Regimens, (100% received at least one anthracycline containing regimen)	
Median	4
Range	1-10

For the Phase 2 part of the study, patients who had no toxicity or in whom toxicity had resolved to Grade 1 or less could receive additional cycles of Rexin-G monotherapy. Two approved protocol amendments permitted an intra-patient dose escalation up to Dose Level 3 for patients who exhibited no toxicity or in whom toxicity had resolved to Grade 1 or less, once adequate safety had been established at the higher dose level. Additionally, each cohort also could be expanded to 6 to 8 patients if significant biologic activity (stable disease or better) was observed at the respective dose level. The principal investigator was allowed to recommend surgical resection/debulking after at least one treatment cycle had been completed.

Objective Response (OR) was evaluated first using the Response Evaluation Criteria in Solid Tumors [RECIST vs 1.0; 25]. Additional evaluations used the International Positron Emission Tomography (PET) Criteria [26] and a modified RECIST assessment, as described by Choi et al. [27]. Safety and efficacy analyses were conducted by the Principal Investigator (S.P.C.).

Clinical objectives/endpoints

The primary objective of the Phase 1/2 study was to determine the safety and clinical toxicity of escalating doses of Rexin-G, as defined by patient performance status, toxicity assessment score, hematologic assessment, and metabolic profiles. The secondary study objectives included (i) evaluation of the potential of Rexin-G infusions to evoke an immune response, recombination events, and/or unwanted vector integration in non-target organs, and (ii) identification of an objective anti-tumor response to Rexin-G monotherapy.

Patient population

The Phase 1/2 study included patients with a pathologic diagnosis

Table 2: Type of Sarcoma (N = 36).

Leiomyosarcoma	10 (27%)
Liposarcoma	6 (16%)
Synovial cell sarcoma	4 (11%)
Osteosarcoma	3 (8%)
MMMT ovary	2 (6%)
Ewing's sarcoma	2 (6%)
Angiosarcoma	2 (6%)
Malignant fibrous histiocytoma	2 (6%)
Chondrosarcoma	1 (3%)
Malignant spindle cell sarcoma	1 (3%)
Fibrosarcoma	1 (3%)
Malignant schwannoma	1 (3%)
Alveolar soft parts sarcoma	1 (3%)

of sarcoma who were refractory to standard chemotherapy (Tables 1-2). Histologic or cytologic confirmation at diagnosis or recurrence was required. Inclusion criteria consisted of an ECOG performance score of 0-1 and adequate hematologic, hepatic, and kidney function. Exclusion criteria included HIV, HBV or HCV positivity, clinically significant ascites, medical or psychiatric conditions that could compromise successful adherence to the protocol, and unwillingness to employ effective contraception during treatment with Rexin-G and for six weeks following treatment completion. The clinical protocol was reviewed and approved by the Institutional Biosafety Committee and the Western Institutional Review Board, Olympia Washington.

Patient recruitment and assignment

The Phase 1/2 study using Rexin-G for sarcoma was registered on www.clinicaltrials.gov (NCT00505713) within one week of study initiation, and patients were recruited on a first come first served basis, after appropriate screening procedures were conducted. Written informed consent, in accordance with the Declaration of Helsinski protocols, was obtained from each patient at the time of enrollment.

Treatment

Rexin-G is an XC-targeted replication incompetent retro vector displaying a high affinity collagen-binding motif on its envelope protein [16] and encoding an N-terminal deletion mutant construct of human cyclin G1 [28] under the control of the Moloney murine leukemia virus long terminal repeat promoter. The Rexin-G vector was produced by transient co-transfection of three separate plasmids in 293T cells (human kidney 293 cells transformed with the SV40 large T antigen) maintained as a fully validated master cell bank [29-30]. The final product exhibited a viral titer of 5 x 10e9 colony forming units (cfu) per milliliter, a biologic potency of 50-70% growth inhibitory activity in A375 melanoma cells, less than 550 bp residual DNA, no detectable E1A or SV40 large T antigen, and no detectable replication competent retrovirus (RCR) [31] . The clinical vector was stored in volumes of 23 ml in 30 ml vials or 40 ml in 150 ml cryobags in a -80+10°C freezer marked Biohazard. Preparation of the Rexin-G vector for patient administration consisted of rapid thawing of the vector in the vial or cryobag in a 34°C water bath. The vector was thawed 15-30 minutes prior to infusion into the patient, and was infused within one hour of thawing, intravenously over 5-10 minutes or at 4 ml/min. All personnel who handled and disposed of the vector observed Bio-safety Level 2 compliance in accordance with the National Institutes of Health Guidelines for Research Involving Recombinant DNA molecules.

The Phase 1/2 sarcoma study enrolled 36 patients who received escalating doses of Rexin-G. Briefly, each treatment cycle was 6 weeks, consisting of 4 weeks of treatment and a 2-week rest period. The following dose levels were employed: Dose Level I = 1 x 10e11 cfu two times a week for 4 weeks (Cumulative dose per cycle (Cum. Dose/cycle: 8 x 10e11 cfu); Dose Level II = 1 x 10e11 cfu three times a week for 4 weeks (Cum. Dose/cycle: 12 x 10e11 cfu); Dose Level III = 2 x 10e11 cfu three times a week (Cum. Dose/cycle: 24 x 10e11 cfu); Dose Level IV = 3 x 10e11 cfu three times a week (Cum. Dose/cycle: 36 x 10e11 cfu); Dose Level V = 4 x 10e11 cfu (Cum. Dose/cycle: 48 x 10e11 cfu). The treatment cycles were repeated if the patient exhibited Grade I or less toxicity.

Safety analysis

Toxicity was assessed before and after each vector infusion, and before beginning an additional treatment cycle. Patients had serum collected for vector antibody detection and peripheral blood mononuclear cells collected for assessment of vector DNA integration and replication competent retrovirus (RCR) at the end of 4 weeks, at 6 weeks, or before the start of a treatment cycle. Toxicity was graded using the National Cancer Institute Common Terminology Criteria Version 3.0 [32]. Evaluation and reporting of adverse events, responses, progression-free survival and overall survival were conducted by the principal investigator (S.P.C.).

Exploratory analysis

Detection of anti-vector antibodies in patients' serum, testing for replication-competent retrovirus (RCR), anti-gp70 *env* antibodies, and vector DNA integration were conducted as described previously [21,22].

Efficacy analysis

Imaging studies were conducted at baseline, 4 weeks and 6 weeks after initiation of therapy, and every 12 weeks (3 months) thereafter. All patients showed either recurrence or progressive disease within 3 months from the last chemotherapy regimen. Tumor response was evaluated using the standard NCI RECIST vs. 1.0 criteria [25], the International PET criteria [26], and the CHOI criteria [27], according to FDA-approved protocols for tumor response assessment. All PET/CT images were performed and reviewed by independent radiologists of the Medical Imaging Center of Southern California, Inc., Santa Monica, U.S.A., who are experts at nuclear and PET imaging and who were blinded to the Rexin-G dose levels.

PET Criteria: The modified International PET Criteria defines a complete response (CR) as disappearance of FDG avid uptake in target and non-target lesions with no new lesions; partial response (PR) as a decrease in SUV max of greater than 25% from baseline, with no new lesions and no obvious progression of non-target lesions; stable disease (SD) as not meeting the criteria for CR, PR or PD, with no symptomatic deterioration attributed to tumor progression; and progression of disease (PD) as an increase in SUV max of greater than 25% from baseline measurements, appearance of any new lesions, and/or obvious progression of non-target lesions [26].

Table 3: Grade 3 Nonserious, Unrelated Adverse Events Reported in ≥2 Patients, by Dose Level.

MedRA System Organ Class/Preferred Term	Dose Level					
	I	II	III	IV	V	Total Grade 3
	N = 6	N = 7	N = 7	N = 8	N = 8	N = 36
Blood and lymphatic system disorders						
Anaemia	4	2	2	1	1	10
Gastrointestinal disorders						
Abdominal pain	2		1			3
Hepatobiliary disorders						
Hyperbilirubinaemia	1	1				2
Hypoalbuminaemia	1			1		2
Investigations						
Blood alkaline phosphatase increased	2	1				3
Metabolism and nutrition disorders						
Hypoalbuminaemia		1	2			3
Hypocalcaemia		2	1			3
Hypokalaemia	2	2			1	5
Hyponatraemia	2	1	2			5
Musculoskeletal and connective tissue disorders						
Musculoskeletal chest pain	1		1			2
Respiratory, thoracic and mediastinal disorders						
Respiratory acidosis	1				1	2
Respiratory failure				1	1	2

Note: Numbers shown are the number of patients who experienced the indicated event at the indicated dose level

CHOI Criteria: The modified CHOI Criteria defines complete response (CR) as the disappearance of all disease and no new lesions; partial response (PR) as a decrease in size of >10% or a decrease in CT density (HU) >15% with no new lesions and no obvious progression of non-measurable disease; stable disease (SD) as not meeting the criteria for CR, PR or PD and no symptomatic deterioration attributed to tumor progression; and progression of disease (PD) as an increase in unidimensional tumor size of >10% which did not meet criteria for PR by CT density, and any new lesions, including new tumor nodules in a previously cystic tumor [27].

Statistical analysis

Frequency tables, graphs, and summary statistics were used to describe patient characteristics and outcome data. Clinical data from August, 2007 to February, 2011 were analyzed. Progression-free survival (PFS) time was approximated, using the times of patient evaluations. OS and PFS times were compared in groups of patients treated at different dose levels, using permutation tests on the log rank statistic with at least 10,000 replications. Tumor response data by different specific criteria (RECIST vs. 1.0, PET or CHOI criteria) were reported. Reported p-values are two-sided, and $p < 0.05$ was considered statistically significant. Analysis was done using NCSS software (Number Cruncher Statistical Systems, Kaysville, Utah). Statistical analysis was performed by a biostatistician not otherwise involved in the study (W.C.B.).

Results

Patient demographics

Table 1 shows the patient demographics. Ninety-seven percent of patients had metastatic disease, a median of 4 previous chemotherapy regimens, and ECOG score of 1. All patients (100%) received at least one anthracycline containing regimen. There were thirteen different types of sarcomas enrolled in the Phase 1/2 study, including leiomyosarcoma (n=10), liposarcoma (n=6), synovial cell sarcoma (n=4), osteosarcoma (n=3), mixed malignant Mullerian tumor of ovary (n=2), Ewing's sarcoma (n=2), angiosarcoma (n=2), malignant fibrous histiocytoma (n=2), chondrosarcoma (n=1), malignant spindle cell sarcoma (n=1), fibrosarcoma (n=1), malignant schwannoma (n=1), and alveolar soft parts sarcoma (n=1) (Table 2).

Analysis of safety

There was no dose-limiting toxicity at the highest dose level given. All 36 patients experienced one or more nondrugs related non-serious AEs during the treatment period, and the majority of these unrelated AEs were Grade 1 or 2. Twenty patients experienced serious adverse events (SAEs) at some time during the treatment, all of which were deemed "not related" to the study drug. Table 3 shows the Grade 3, non-serious, non-drug related adverse events, the most frequent of which included anemia (10 patients), hypokalemia (5 patients), and hyponatraemia (5 patients). Abdominal pain, blood alkaline phosphatase increase, hypoalbuminemia, and hypocalcaemia were reported in 3 patients each. Hyperbilirubinaemia, musculoskeletal chest pain, respiratory acidosis, and respiratory failure were reported in 2 patients each. All other Grade 3 AEs were reported in only 1 patient each and all were due to disease progression. There was no Grade 3 neutropenia, fever/neutropenia, nor nausea/vomiting reported in the study patients. No relationship was apparent between

Table 4: Listing of Patients with Drug-Related Adverse Events.

Dose Level	MedDRA System Organ Class	MedDRA Preferred Term	Toxicity Grade
0	General disorders and administration site conditions	Chills	1
0	General disorders and administration site conditions	Fatigue	2
0	General disorders and administration site conditions	Fatigue	2
1	General disorders and administration site conditions	Fatigue	1
1	General disorders and administration site conditions	Fatigue	1
3	Immune system disorders	Hypersensitivity	1
4	General disorders and administration site conditions	Chills	1
4	General disorders and administration site conditions	Fatigue	1

Notes: all drug-related AEs were nonserious; toxicity grade according to NCI-CTCAE

AEs and dose of Rexin-G administered. In fact, there were more non-related Grade 3 adverse events in patients who received lower doses of Rexin-G, suggesting that the adverse events were related to the cancer symptomology. Correlative analysis showed no vector neutralizing antibodies detected in serum, and there was no evidence of vector DNA integration nor replication-competent retrovirus in peripheral blood lymphocytes, further attesting to the overall safety of Rexin-G.

Eight patients experienced drug related adverse events; all were mild or moderate in severity (Table 4). These included Grade 1-2 fatigue in 5 patients, Grade 1 chills in 2 patients and Grade 1 rash (hypersensitivity) in one patient.

Analysis of efficacy

Evaluation of tumor response used a modified RECIST vs. 1.0, PET, and CHOI Criteria, which compared responses seen at Weeks 4 and 6 with baseline responses; subsequent responses beyond Week 6 were compared with responses seen at Week 6. The rationale for the modified RECIST vs. 1.0 [30,33] is based on the known Rexin-G mechanism of action [33-37]. Briefly, Rexin-G blocks the cell cycle in G1 phase and causes apoptosis and necrosis of tumors, without causing bone marrow suppression. Consequently, Rexin-G may evoke an inflammatory reaction to tumor debris and white cell migration to necrotic tumors, as well as tumor lymphocyte infiltration as part of the body's natural clean-up process [22,33]. These immune-related reactions may cause the tumors to look larger and occult lesions to become more apparent as new lesions. These features represent pseudo-progression events, now recognized and incorporated in immune-related RECIST (irRECIST) criteria in many cancer immunotherapy protocols [38]. Further, there was a 4-week chemotherapy wash-out period prior to initiation of Rexin-G treatment; hence, the tumors would be expected to be rapidly progressing, and tumor control may not be immediately obtained, until 4-6 weeks of Rexin-G treatment. Therefore, it was considered appropriate to compare tumor responses observed beyond Week 6 to those at Week 6 instead of untreated baseline measurements.

Of the 36 enrolled and treated patients, 6 were treated at Dose Levels I-II, 7 were treated at Dose Levels II-III, 7 were treated at Dose Level III, 8 were treated at Dose Level IV, and 8 were treated at Dose Level V. Thirty-three patients received at least one complete cycle (4 weeks) of treatment and had a follow-up PET-CT scan and were considered evaluable for efficacy. By RECIST, 21 patients had SD and 12 had PD. By International PET criteria, 9 patients achieved a PR, 21 had SD, and 3 had PD. By the modified RECIST criteria of Choi et al., 8 patients achieved a PR, 23 had SD, and 2 had PD. The tumor control rates (CR + PR + SD) were 64% (22/33 patients) by RECIST vs. 1.0; 91% (30/33) by PET criteria and 94% (31/33) by Choi-modified RECIST. There were more PRs observed using PET and Choi-modified RECIST criteria, indicating that these measurements are more sensitive than RECIST vs. 1.0 as clinical indicators of early tumor responses to Rexin-G treatment.

In the 33 evaluable patients, a significant dose-response relationship was clearly observed between overall survival and the Rexin-G dosage ($p < 0.002$). Specifically, none of the patients who received the lowest dose of Rexin-G survived one year. In contrast, 28.5% of patients who received Dose Levels II-III were alive at one year after Rexin-G treatment initiation, although none of the patients receiving Dose Levels II-III survived two years. The best survival data was observed in patients who received the highest doses (Dose Levels IV-V) of Rexin-G, i.e., overall survival was 38.5% at one year and 31.0% at two years. Likewise, in the 36 enrolled patients, overall survival was 31.2% at one year and 25.0% at two years. As of the last follow-up on November 11, 2015, one patient with malignant schwannoma metastatic to lungs was still alive, with no disease progression, 7 years from Rexin-G treatment initiation. This patient received no further anti-cancer treatment after discontinuation of Rexin-G treatment in June, 2010 (Table 5).

Discussion

Rexin-G is an XC/tumor-targeted retro vector displaying a high affinity XC-protein binding motif on its surface envelope and bearing a cytocidal dominant-negative cyclin G1 construct as its genetic payload. When injected intravenously, Rexin-G, with its active tumor targeting function, seeks out and accumulates in the TME, wherein XC-proteins are found in abundance as a result of tumor invasion, neoangiogenesis, and/or extracellular matrix remodeling, thus increasing the effective local concentration in tumors in the vicinity of target cells [18]. Rexin-G causes cell death by blocking the cell cycle at G1 phase, and inducing apoptosis of proliferative cancer cells and associated neovasculature, as was demonstrated in preclinical studies [34-37], and later confirmed in the clinical setting [18,23].

In this Phase 1/2 sarcoma study, tumor control was demonstrated by a significantly longer duration of survival gained in the higher dose cohorts compared to the low dose cohorts. A significant proportion of patients exhibited partial responses (PR) and stable disease (SD) by PET and CHOI criteria, compared to RECIST, suggesting increased sensitivity of PET and CHOI criteria for detection of early

Table 5: Summary of Objective Response, Disease Control Rate, Progression-free Survival and Overall Survival in Evaluable Patients.

Category N = 33	Dose Level I N = 6	Dose Level II-III N = 14	Dose Level IV-V N = 13
OR			
RECIST	3SD, 3PD	10 SD, 4 PD	8SD, 5PD
PET	1PR, 4SD, 1PD	5 PR, 8SD, 1PD	3PR, 9SD, 1PD
CHOI	1PR, 5SD	6 PR, 7SD, 1PD	1PR, 11SD, 1PD
DCR			
RECIST	3/6 (50%)	10/14 (71%)	8/13 (62%)
PET	5/6 (83%)	13/14 (93%)	12/13 (92%)
CHOI	6 (100%)	13/14 (93%)	12/13 (92%)
PFS, mos.			
RECIST	1.2	3.8	4.1
PET	2.8	5.3	3.25
CHOI	4.2	5.3	3.25
Median OS, mos.	3.3	7.8	11.5
1 yr. survival	0%	28.5%	38.5%
2 yr. survival	0%	0%	31.0%
Dose Response Relationship (OS)			p = 0.002

RECIST: Response Evaluation Criteria In Solid Tumors; PET: Positron Emission Tomography; CHOI: modified RECIST; PFS: Progression-free Survival; OS: Overall Survival; Dose Level I = 1 x 10e11 cfu twice per week (BIW); Dose Level II = 1 x 10e11 cfu three times per week (TIW); Dose Level III = 2 x 10e11 cfu TIW; Dose Level IV = 3 x 10e11 cfu TIW; Dose Level V = 4 x 10e11 cfu TIW

tumor responses to Rexin-G treatment. Moreover, the infiltration of leukocytes into tumors seen following Rexin-G treatment, in the absence of immune suppression, highlights the inadequacy of limited morphological assessments. No cumulative toxicity was observed with prolonged use of Rexin-G up to one year of treatment. Correlative analysis showed no vector neutralizing antibodies detected in serum, and there was no evidence of vector DNA integration nor replication-competent retrovirus in peripheral blood lymphocytes, further attesting to the overall safety of Rexin-G. Surgical resection of residual tumors following Rexin-G treatment-which enabled histological examination and strategic tumor debulking-followed by the administration of additional post-operative treatment, serves to underscore the potential clinical benefit of Rexin-G in the neoadjuvant/adjuvant settings [18, 23].

In conclusion, the primary and secondary objectives of this Phase 1/2 study have been met. The overall safety of Rexin-G, administered within the defined dose ranges, was clearly established by the absence of dose limiting toxicity or vector safety concerns. A dose response improvement in overall survival was apparent with Rexin-G usage. Indeed, the significant dose-dependent survival advantage-ranging from 0% (at Dose Level I) to 38.5% at 1 year and 31% at 2 years (at Dose Level IV-V) following initiation of Rexin-G monotherapy-not only serve to define an optimal dosing regimen for Rexin-G, but would also constitute the gold standard in evaluating Rexin-G efficacy if pivotal Phase 3 clinical trials for chemotherapy resistant bone and soft tissue sarcoma were to be conducted.

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